Evaluation of antibacterial and antioxidant activity of fruits extract of *Argemone mexicana* Linn.

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Abstract

The in vitro antibacterial and antioxidant properties of 50% methanolic extract of fruits from *Argemone mexicana* Linn was investigated. In this study the antibacterial activity of *Argemone mexicana* Linn against different gram positive and gram negative bacteria by disc diffusion method and Antioxidant activity by Fenton reaction was undertaken. *Argemone mexicana* Linn has shown effective against Gram positive bacteria and Gram negative bacteria and the zone of inhibition was measured. In another experiment antioxidant activity was observed and IC₅₀ was shown at µg concentration. These results are important because this plant is widely used for medicinal and ornamental purpose.

KEY WORDS: Argemone mexicana, antibacterial, antioxidant

Introduction

*Argemone mexicana* Linn is known as Satyanashi which is medium size tree belongs to family Pappoveraceae. A strong branched prickly annual, 60-90 cm in height with yellow latex; leaves simple, sessile and spiny. Flowers large, bright yellow, terminal on the short leafy branches; fruits prickly capsules, oblong-ovoid, opening by 4-6 valves; seeds numerous.¹ The seeds contain 22-36% of pale yellow non-edible oil, called Argemone oil or Katkar oil, which contains the toxic alkaloids sanguinarine and dihydrosanguinarine. The plant contains alkaloids as berberine, protopine, sanguinarine, optisine, chelerytherine etc. The seed oil contains myristic, palmitic, oleic, linoleic acids etc.² According to Ayurveda the plant is diuretic. Purgative and destroys worms. It cures leprosy, skin-diseases, inflammations and bilious fevers.³

An anti-microbial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi, or protozoan’s. Antimicrobial drugs either kill microbes (microbicidal) or prevent the growth of microbes (micro biostatic).⁴ An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation

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reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death. When the chain reaction occurs in a purified monomer, it produces a polymer resin, such as a plastic, a synthetic fiber, or an oil paint film. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols. Although oxidation reactions are crucial for life, they can also be damaging; hence, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidase. Low levels of antioxidants, or inhibition of the antioxidant enzymes, cause oxidative stress and may damage or kill cells.5

Materials and Methods

Plant Material
The fresh fruits of *Argemone mexicana* Linn were collected from Idgah Hills Bhopal and authenticated by DR. Promoad Patil (Botanist) at Gov. M.L.B. Girls (Autonomous) college, Bhopal (M.P.) and Sheet no. of Herbarium is 903.

Preparation of Extract
The fruits of *A. mexicana* was washed with DDW and dried in shade then powdered by grinder. The powder was treated with petroleum ether up to 3 hrs for defatting. 50gm of powder was taken in separating funnel and 50% methanol added then mixed gently. After every 24 hrs extract was collected in a beaker till the solvent appears colorless. Cycle is repeated 3 times. Then extract was dried into powder by water bath at 55ºc and hot air oven at 45ºc. Total weight of extract powder was measured and obtained 16 % yield of extract.6

Test organism
The following gram negative and gram positive bacteria i.e. *Staphylo aureus*, *Pseudomonas aeroginosa*, *Staphylococcus epidermis*, *Shigella flexineri*, *Bacillus subtilis* and *Escherichia coli* were used for antibacterial activity which were received from stock culture of our laboratory (J.N.C.H. & R.C.) Bhopal (M.P.).

Antibacterial activity
Antibacterial activity of 50% methanolic extract from fruits of *Argemone mexicana* was investigated using disc diffusion method. 20 ml of sterile Nutrient agar was added in test tubes after that Petri plates were prepared and cultured were swabbed on the top of the solidified media and allowed to dry. The test was conducted at four different concentration of the crude extract 25%, 50%, 75%, and 100%. (100 µl per disc), the disk which was soaked with extract was placed on the surface of the medium plate and was incubated for 18-24 hrs at 37ºC and zone of inhibition measured by help of scale.7

Antioxidant activity
The hydroxyl radical attacked deoxyribose and initiated a series of reaction that eventually resulted in the formation of Thiobarbituric acid reactive substances (TBARs). The measurement of TBARs thus given an index of free radical scavenging activity. The reaction mixture consists of a deoxyribose (3 Mm, 100 µl),
ferric chloride (Fe 3+ 0.2 mM 50 µl) Ascorbic acid (0.1 mM 100 µl ) and H2O2 (100 mM µl), stock solution of *Argemone mexicana* fruits extract 10 mg/ml were prepared from which 10-100 µl were added in reaction mixture. The final volume was made up to 1 ml by adding adequate quantity of phosphate buffer saline (pH=7.4) and incubated for 1 hrs at 37° C. The reaction was stopped by adding 0.5 ml of 5 % TCA and 0.5 ml of 1 % TBA. The mixture was than incubated for 20 min in a boiling water bath. The absorbance was measured at 532 nm. Ascorbic acid was used as a positive control. The result is expressed as the % inhibition of TBARs. Antioxidant activity

The in vitro antioxidant activity of *Argemone mexicana* fruits extract was tested in various concentrations against Ascorbic acid as standard. Percentage of TBARS is calculated for both Ascorbic acid and *Argemone mexicana* fruits extract, with the help of formula, for a comparative study. The percentage of TBARS was plotted in the graph in different concentration.

\[
\text{IC}_{50} = \frac{A_c - A_t}{A_c} \times 100
\]

\(A_c = \text{Absorbance of Control}\)
\(A_t = \text{Absorbance of test}\)

The antioxidant activity of the *Argemone mexicana* hydromethanolic fruits extract was monitored by Fenton method. The obtained result showed that the Fruits extract exhibited significant degree of in vitro antioxidant activity, when compared to standard (ascorbic acid), although less than the standard. And IC\(_{50}\) of *Argemone mexicana* extract was found 49.38.

The result obtained present study indicated that *Argemone mexicana* fruits extract exhibits free radical scavenging activity. The overall antioxidant activity of *Argemone mexicana* fruits extract might be attributed to its polyphenolic content and other phytochemical constituents. The finding of the present study suggests that *Argemone mexicana* could be a potential source of natural antioxidant that could have greater importance as therapeutic agent in preventing or slowing oxidative stress related degenerative diseases.  

Results and Discussion

Antibacterial activity

Present study showed that hydromethanolic extract of *Argemone mexicana* fruits caused antimicrobial activity against gram positive and gram negative bacteria but it was more effective against gram negative bacteria. Antibacterial activity of *Argemone mexicana* was reported on *klebsiella oxytoca*, *Vibrio damsella*, *enterobactor aerogenes* and *E. Coli*. The current result has supplemented earlier report on growth inhibitory effect on solvent extract of *Argemone mexicana* on few other species of pathogenic bacteria. Therefore, it is evident that the plant contains active ingredients flavanoid and terpenoid are also known to possess astringent and antimicrobial property. This seems to be responsible for growth of inhibition of bacteria and increase antimicrobial property. 

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Conclusion
From the present investigation it may be concluded that hydromethanolic extract of *Argemone mexicana* Linn has potent antibacterial activity against gram positive and gram negative bacteria. Hydromethanolic extract of *Argemone mexicana* Linn showed good antioxidant activity and IC50 was found 49.38 at 70μg/ml concentration. Further studies are however needed to isolate the active molecule responsible for both antimicrobial and antioxidant activity. The studies thus may lead to the formulation of a potent antimicrobial and antioxidant agent.

References

Table 1: Antibacterial Activity of *Argemone mexicana* fruits extract against bacterial strains:

<table>
<thead>
<tr>
<th>Name of organisms</th>
<th>% conc. of extract (zone of inhibition (mm))</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>25%</td>
</tr>
<tr>
<td><em>Streptococcus aureus</em></td>
<td>---</td>
</tr>
<tr>
<td><em>Streptococcus epidermidis</em></td>
<td>---</td>
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<tr>
<td><em>Bacillus subtilis</em></td>
<td>---</td>
</tr>
<tr>
<td><em>Shigella flexineri</em></td>
<td>6.5</td>
</tr>
<tr>
<td><em>E.coli</em></td>
<td>7.0</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>6.0</td>
</tr>
</tbody>
</table>

Figure 1: Antimicrobial activity of extracts of *Argemone mexicana* fruits in different Concentration
Figure 2 - Zone Of Inhibition of *Argemone mexicana* fruits extract against different Gram Positive and Gram Negative Bacteria
Table-2 In-vitro antioxidant activity of extract of *Argemone mexicana* fruits vs standard Ascorbic acid

<table>
<thead>
<tr>
<th>S. N</th>
<th>Conc. of Ascorbic acid (µg/ml)</th>
<th>%TBARS</th>
<th>Conc. of Drug (µg/ml)</th>
<th>%TBARS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>10</td>
<td>52.10</td>
<td>10</td>
<td>4.93</td>
</tr>
<tr>
<td>2.</td>
<td>20</td>
<td>75.20</td>
<td>20</td>
<td>14.81</td>
</tr>
<tr>
<td>3.</td>
<td>30</td>
<td>111.40</td>
<td>30</td>
<td>17.28</td>
</tr>
<tr>
<td>4.</td>
<td>40</td>
<td>134.80</td>
<td>40</td>
<td>20.98</td>
</tr>
<tr>
<td>5.</td>
<td>50</td>
<td>157.50</td>
<td>50</td>
<td>33.33</td>
</tr>
<tr>
<td>6.</td>
<td>60</td>
<td>158.70</td>
<td>60</td>
<td>43.20</td>
</tr>
<tr>
<td>7.</td>
<td>70</td>
<td>159.30</td>
<td>70</td>
<td>49.38*</td>
</tr>
<tr>
<td>8.</td>
<td>80</td>
<td>173.20</td>
<td>80</td>
<td>53.08</td>
</tr>
<tr>
<td>9.</td>
<td>90</td>
<td>187.20</td>
<td>90</td>
<td>55.55</td>
</tr>
<tr>
<td>10.</td>
<td>100</td>
<td>227.90</td>
<td>100</td>
<td>72.83</td>
</tr>
</tbody>
</table>

IC50=Concentration at which % inhibition of TBARS is 50%, *denoted the IC50 value in the drug

Figure3:- In vitro Antioxidant Activity of *Argemone mexicana* fruits extract

Figure4:- In vitro Antioxidant activity of Standard Ascorbic Acid